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Performance and Meat Quality of Broilers Infected with *Escherichia coli* and Administered with Bio Additive, Probiotic, and Antibiotic

L. Istiqomah[a,*], S. N. Hayati[a], E. Damayanti[a], H. Julendra[a], A. A. Sakti[a], & T. Untari[b]
[a]Research Unit for Processes Development & Chemical Engineering (UPT. BPPTK), Indonesian Institute of Sciences (LIPI)
Jln. Jogja - Wonosari Km. 31, Gading, Playen, Gunungkidul, Yogyakarta 55861, Indonesia
[b]Laboratory of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University
Sekip Unit II, Yogyakarta 55281, Indonesia
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ABSTRACT

An experiment was conducted to determine the efficacy of bio additive administration (a mixture of *Lumbricus rubellus* extract, *Morinda citrifolia* leaves extract and lactic acid bacteria), probiotic, and antibiotic to the performance and meat quality of broiler infected with Avian Pathogenic *Escherichia coli* (APEC). In this study, 140 Jumbo 747 unsexed one-day old chicks were distributed randomly into 20 units of cages, each filled with 7 broilers. Twenty cages were assigned into 5 treatment groups, each treatment in 4 equal replicates. The treatments were as follows: A= *E. coli* infection (positive control), B= *E. coli* infection + bio additive, C= *E. coli* infection + probiotic, D= *E. coli* infection + antibiotic, E= No *E. coli* infection (negative control). A commercial corn-soybean-based broiler diet was formulated as the basal diets. The experimental period was 35 d and at 21st d of age the broilers were infected with *E. coli* except the E treatment. The result showed that bio additive administration (B) increased the final body weight (1,659.52 g) and body weight gain (1,616.81 g) and resulted in less FCR (1.87) among other treatments. The lowest mortality rate was recorded in B treatment (3.57%) and D treatment (3.57%). Probiotic (C treatment) and antibiotic (D treatment) decreased (P<0.05) meat pH and tenderness compared to other treatments. Meanwhile bio additive administration did not affect the meat quality (pH, cooking loss, water-holding capacity, tenderness, and fat) compared to positive and negative controls. The lowest meat cholesterol content was observed in B treatment (54.02 mg/100 g). It is concluded that bio additive administration on broiler infected with *E. coli* increased the broiler performance and decreased the meat cholesterol compared to other treatments.

Key words: bio additive, probiotic, antibiotic, E. coli, broiler performance

ABSTRAK

Penelitian dilakukan untuk mempelajari pengaruh pemberian bio aditif (campuran ekstrak cacing tanah *Lumbricus rubellus*, ekstrak daun mengkudu *Morinda citrifolia* dan bakteri asam laktat), probiotik, dan antibiotik terhadap performa dan kualitas daging ayam broiler yang diinfeksi *avian pathogenic Escherichia coli* (APEC). Sebanyak 140 ekor DOC 747 Jumbo didistribusikan secara acak ke dalam 20 unit kandang, masing-masing diisi dengan 7 ekor ayam. Dua puluh kandang tersebut dibagi ke dalam 5 kelompok perlakuan, tiap perlakuan terdiri atas 4 ulangan. Kelompok perlakuan terdiri atas perlakuan A= infeksi *E. coli* (kontrol positif), B= infeksi *E. coli* + bio aditif, C= infeksi *E. coli* + probiotik, D= infeksi *E. coli* + antibiotik, E= tanpa infeksi *E. coli* (kontrol negatif). Pakan komersil berbasis jagung-kedelai diformulasi sebagai pakan basal. Percobaan dilakukan selama 35 hari dan pada hari ke-21 broiler diinfeksi *E. coli* kecuali perlakuan E. Hasil penelitian menunjukkan bahwa pemberian bio aditif (B) menghasilkan berat badan akhir (1.659,52 g) dan pertambahan bobot badan (1.616,81 g) yang lebih tinggi (P<0,05) serta FCR yang lebih efisien (1,87) dibandingkan perlakuan lainnya. Tingkat kematian terendah tercatat pada perlakuan B (3,57%) dan D (3,57%). Pemberian probiotik dan antibiotik menurunkan (P<0,05) pH dan keempukan daging dibandingkan dengan perlakuan lainnya. Sementara itu, pemberian bio aditif tidak mempengaruhi kualitas daging (pH, susut masak, kapasitas menahan air, keempukan, dan kadar lemak daging) dibandingkan dengan kontrol positif dan negatif. Kolesterol daging terendah tercatat pada perlakuan B (54,02 mg/100 g). Dapat disimpulkan bahwa pemberian bio aditif pada broiler yang diinfeksi *E. coli* dapat meningkatkan performa ternak dan menurunkan kolesterol karkas dibandingkan perlakuan lainnya.

Kata kunci: bio aditif, probiotik, antibiotik, E. coli, performa broiler

*Corresponding author:
E-mail: lust001@lipi.go.id / ps_uty@yahoo.com

INTRODUCTION

Avian colibacillosis is an infectious disease of birds caused by avian pathogenic *E. coli* (APEC), which is considered as one of the principal causes of high morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions, either as primary pathogen or as a secondary pathogen (Kabir, 2010). Due to prohibition of antibiotics application as growth promoters (AGP's) and sub therapeutic use in animal feed industry since 2006 in European Union (EU) and the possibility of a ban in other countries, the importance of functional natural materials in poultry diets have increased attention in recent years. The natural materials may replace the AGP's are pre- and probiotic, bacteriocins, organic acids, essential oils, herbs and spices (plant extracts), yeast cultures, oligosaccharides, and flavourings (Gaggia *et al*., 2010). Earthworm (*Lumbricus rubellus*) meal is one of natural feed additive used for this purpose. Cho *et al*. (1998) reported the antibacterial activity of earthworm *L. rubellus* in broad spectrum against some pathogenic bacteria due to its bioactive compound 'lumbricin I' and chemotherapeutic components of lumbrokinase and fibrinolytic enzymes.

There is global interest in harnessing bioactive properties of plants and their secondary compounds as alternatives to chemical, drugs and growth promoters (Durmic & Blache, 2012). Noni (*Morinda citrifolia*) leaf is an herbal plant containing anthraquinone compound as an antibacterial. This compound has been shown to fight against infectious bacteria strains such as *Pseudomonas aeruginosa, Proteus morgaii, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella,* and *Shigela* (Deshmukh *et al*., 2011).

A number of new probiotic strains of lactic acid bacteria have been developed and have benefits to animal and human health, including increased protection against intestinal pathogens and modulate the immune system (Crittenden *et al*., 2005). Probiotic enhanced the systemic antibody response to some antigens in broiler chickens (Haghigi *et al*., 2005), promoted the poultry meat quality (Kabir, 2009), increased the body weight, decreased feed conversion ratio, and reduced coliforms number in the ileum (Taheri *et al*., 2010).

This study focused on determining the effect of drinking water administered by earthworm extract, noni leaves extract and lactic acid bacteria (bio additive) on growth performance and meat quality of broiler infected with Avian Pathogenic *Escherichia coli* (APEC).

MATERIALS AND METHODS

Bio Additive

Bio additive material contained earthworm meal extract, noni (*M. citifolia*) leaves extract, and dry culture of lactic acid bacteria.

Preparation and extraction of earthworm meal. Preparation of earthworm meal refers to the modified methods of Edwards (1985). Earthworms were separated from the media and then washed with water to remove dirt and grime on the outer skin digestive tract (fecal mud) of the worm. Then worms soaked in cold water 4 ^{o}C for 24 h. Formic acid 80% was added as much as 3% by weight of the worms. The worm was milled using a blender to become a paste and the paste was dried in an oven at 50 ^{o}C for 12 h and sieved to obtain a homogeneous particle size of ± 40 mesh. Earthworm extract was prepared by the dekokta extraction method with water at 90 ^{o}C for 30 min (Ministry Health of RI, 2000). One part of the earthworm meal and 10 parts of water were heated and then filtered using a filter cloth. The filtrate was concentrated by evaporation until a thick consistency.

Preparation and extraction of noni leaves powder. Mature noni leaves were collected from Playen Gunungkidul area, then washed and dried in an oven at 50-60 ^{o}C, ground and sieved to 30 mesh of particle size. Leaves powder was soaked in 40% ethanol for 3 d with occasional stirring, placed in a cool place, and protected from the sun light. Filtrate obtained was evaporated over a water bath and blew with a fan until a thick consistency material was obtained.

Granulation of noni leaves and earthworm meal extracts. The granulation of noni leaves and earthworm meal extracts was performed by wet granulation method. Thick crude extract of earthworm meal were blended with drying agent, Manihot starch. Then, the second active ingredient (noni leaves extract) was added. Sucrose was added into the mixture as a filling agent. The last material added into the mixture was carboxymethyl cellulose (CMC-Na) as a suspending agent. The wet mass sieved to 20 mesh of particle size and dried at 40-60 ^{o}C for 24-48 h. The dry mass then sieved again to 20 mesh of particle size.

Preparation of dry cultures of lactic acid bacteria. Probiotic was prepared by microencapsulation methods using spray dryer (Lab Plant SD-Basic) according to Barbosa-Canovas (2005). Probiotic selected isolate (*Pediococcus acidilactici* RO1) was cultivated in de Man Rogosa Sharpe (MRS) Broth media at 37 ^{o}C for 18 h. The culture was centrifuged at 4500 rpm for 10 min, then the biomass / pellet was mixed with skim solution (20% w/v) and gum arabic (1% w/v). The solution was homogenized using a homogenizer at 8000 rpm for 5 min before microencapsulation process. Spray dryer operating conditions as follows: inlet air temperature 110 ^{o}C, outlet air temperature 68 ^{o}C, and the speed of pump 3. Dried cultures of probiotic that obtained from spray dryer then added with skim as fillers and adjusted to $1x10^{9}$ cfu/g of cell density.

Formulation of bio additive. Earthworms extract, noni leaves extract, starch, sucrose, and CMC-Na (200 : 200 : 200 : 2,836 : 564 mg/kg BW) were mixed homogenously and then be mixed with dried cultures of probiotic ($1x10^{9}$ cfu/g) to produce granule form called bio additive. Dose of bio additive used in this study was 4 g/kg BW.

Antibiotic oxytetracycline used in this study was in a mixed form with multivitamins (vitamin A, D3, E,

K3, B1, B2, B6, B12, and C), nicotinic acid, and calcium-D-pantothenate. Dose of antibiotic used was 0.71 g/L of drinking water. Probiotic applied in this study contained 1x10^{12} cfu/g of *Lactobacillus acidophillus*, *Bacillus subtilis*, *Bacillus megaterium*, *Lactobacillus bulgaricus*, *Candida pintolopesii*, *Saccharomyces cerevisiae*, *Aspergillus oryzae*, and *Streptococcus thermophillus*. Dose applied was 0.5 ml/L of drinking water.

Experimental Diet

Basal diet composed of rice bran, pollard, fish meal, crude palm oil, calcium carbonate ($CaCO_3$), di calcium phosphate (DCP), lysine, DL-methionine, commercial premix and formulated to meet nutrient requirements of broilers as recommended by NRC (1994) (Table 1). The protein level of the diets was approximately 22%-23%. Nutrient content of diet based on laboratory analyses.

Determination of *E. coli* Dose

To determine the infectious dose-50 (ID_{50}), 20 broiler chicks were kept separately from others from DOC. In the early stages DOC intracardiac blood samples were taken to ensure the DOC were free from *E. coli*. *Escherichia coli* used in this study was Avian Pathogenic *Escherichia coli* (APEC). At 3-wk old, the broilers were infected with *E. coli* orally with a serial doses of 10^6, 10^7, 10^8, 10^9 cfu/ml/bird and then kept for 14 d to observe clinical symptoms and the death of chickens. *E. coli* was isolated from died chickens or that indicating clinical symptoms to confirm the cause of the disease (Radji *et al*., 2003). Infection dose (ID_{50}) was determined by modified method of Reed & Muench (1938).

Table 1. Composition and nutrient content (DM basis) of the basal diets used in the experiment

Ingredients	Composition (%)		
Ground yellow corn	62.55		
Rice bran	1.00		
Pollard	3.25		
Soybean meal	23.00		
Fish meal	7.50		
Crude palm oil	1.00		
CaCO3	0.50		
DCP	0.10		
L-lysine	0.80		
DL-methionine	0.27		
Commercial premix	0.03		
Total	100.00		
Nutrient content:	Broiler diets	Broiler diets requirements[1)]	
	Starter-Finisher	Starter	Finisher
Moisture (%)	10.91	10.00	10.00
Crude protein (%)	22.68	23.00	20.00
Fat (%)	4.48	max. 7.40	max. 8.00
Crude fiber (%)	2.56	max. 6.00	max. 6.00
Ash (%)	4.62	max. 8.00	max. 8.00
Ca (%)	1.99	1.00	0.90
P (%)	0.38	0.50	0.40

1) National Research Council (1994)

In Vivo Experiment

A total number of 140 Jumbo 747 unsexed one-day old broilers with initial body weight of 42-43 g were allocated randomly into 5 treatments. All birds kept under a similar condition of management throughout the experimental period lasting for 35 d of age. Initial brooding temperature was 33 °C in the first week of age and reduced gradually 2 °C per wk to 24 °C. Diets and water were provided *ad libitum* all over the experimental period. Nutrient content of the experimental diet is presented in Table 1. The experiment was designed in a completely randomized design with 5 treatments, with 4 equal replicates and 7 broiler chicks each. The treatments tested on broiler chickens were as follows: A= *E. coli* infection (positive control), B= *E. coli* infection + bio additive, C= *E. coli* infection + probiotic, D= *E. coli* infection + antibiotic, E= No *E. coli* infection (negative control).

Bio additive, probiotic, and antibiotic were given every day through drinking water. Vaccinations were given at the age of 4 d old (ND-IB vaccine, Newcastle Disease, Infectious Bronchitis-eye drops), 10 d old (IBD vaccine, Infectius Bursal Disease), and 15 d old (ND Lasota vaccine, Newcastle Disease-oral). At the age of 21 d old (6 d post-vaccination ND Lasota) the *E. coli* was infected to broilers orally with dose 10^8 cfu/ml/bird (based on ID_{50}). The appearance of clinical symptoms was observed one week after infection. Necropsy was conducted on the chicks with the symptom to see macroscopic (PA) and microscopic (histopathology) changes, and blood profiles. At the end of experimental period, twenty chickens (4 chickens per treatment) were randomly selected, weighed, and slaughtered to evaluate the meat quality.

Growth Performance and Meat Quality of Broilers

Growth performance. Feed intake was recorded daily by subtracting the amount of offered feed with the residual feed for each replicate. Final body weight was assessed basis from initial day to the final day of the experiment. Mortality was recorded daily, and percentage of mortality was calculated. Feed conversion ratio (FCR) was calculated as total feed intake divided by final body weight of live chicken (Timmerman *et al*., 2006).

Meat quality. At the end of experiment (35 day of age) one bird per replication were randomly slaughtered for meat quality analysis (pH, cooking loss, water holding capacity, tenderness, fat, and cholesterol content). The breast muscles (without skin) were used for physical quality analysis (pH, cooking loss, water holding capacity, and tenderness), while the thigh muscles were used for fat and cholesterol determination. The meat pH was measured according to Soewedo (1994). The meat

samples were mashed with meat grinder, as much as 2 g sample diluted with 18 ml of distilled water and stirred until homogeneous then filtered. The filtrate samples were measured with a pH meter (HI 9811X Piccolo, Hanna instrument).

Cooking loss was determined according to Nikmaram *et al*. (2011). Meat samples (20 g) were placed in polyethylene plastic, then sealed with vaccumpack, and heated in a water bath at 80 °C for 30 min. After cooked, samples were cooled at room temperature, dried surface with filter paper, reweighed using an analytical balance (Metler AE100-0.001), and the cooking loss calculated from differences in raw and cooked weight.

Water holding capacity was determined according to Hamm method (1960). Meat samples (0.3 g) were placed on Whatman 41 filter paper between two metal plates with a pressure load of 35 kg for 5 min until wet area formed on the filter paper. Wet area was calculated by subtracting the area covered meat samples (in the area of a circle) of the total area (wide outer ring).

Tenderness was measured according to Soeparno *et al*. (2005). Meat samples were sealed in polypropylene plastic, then heated in a water bath at 80 °C for 30 min. After cold, samples were made with a size of 1.5 x 0.67 cm or tubular shape, and placed on Wanner-Blaztser Shear Force, Model Salter 235. Samples were cut parallel to the muscle fiber direction and measurement result was noted. Fat content of the breast was measured by extraction in a Soxhlet apparatus with petroleum ether (AOAC, 1990). Total fat were extracted from the samples (about 4 g) with 40 ml chloroform:methanol (2:1, vol/vol) in a 50 mL ground-glass extraction flask according to the method of Folch *et al.* (1957). The meat cholesterol content was determined by Liebermann-Burchad reaction (Kenny, 1952) using a spectrophotometer at a wavelength of 420 nm. Coloring reagent used were acetic acid anhydride and concentrated sulfuric acid in different solvents such as chloroform or ether.

Statistical Analysis

The effect of treatments on broiler performance and meat quality were evaluated using the analysis of variance of a completely randomized design and the differences among mean treatments were analyzed using Duncan's Multiple Range Test (Gomez & Gomez, 2007).

RESULTS AND DISCUSSION

Broilers Performance

The initial live weight indicated that DOC were distributed well within the experimental treatments. There were no difference on body weight, weight gain, feed intake, FCR, and mortality between infected (A treatment) and uninfected broilers (E treatment) (Table 2). The changes in body weight, weight gain and FCR were not good indicators of the infection of *E. coli*. Teo & Tan (2006) reported the similar result that *E. coli* challenged broilers did not indicate changes in body weight, weight gain, and FCR.

Broilers supplemented by bio additive (B treatment) had the highest final body weight and body weight gain ($P<0.05$) and the lowest feed conversion ratio. Bio additive might have growth promoter effect and reduce the negative effect of the presence of *E. coli* in broiler. Teo & Tan (2006) observed an increasing trend in weight gain and improved FCR of broilers challenged by *E. coli* and supplemented by *B. subtilis* (probiotic) compared with those in antibiotic. Alkhalf *et al*. (2010) reported that 0.8-1.0 g/kg diet of probiotic supplementation significantly increased the body weight and daily weight gain of broiler chicks for growth period of 3–6 wk and also improved feed conversion. Taheri *et al*. (2010) reported that *P. acidilactici* supplementation (10^8 cfu/g diet) on broilers diet increased body weight and decreased FCR ($P<0.05$). Ton *et al.* (2009) reported that chicken fed diet supplemented with 2% red worm had the highest live weight at 10 wk (1,925 g) and better FCR (2.95). Sofyan *et al.* (2010) confirmed that earthworm meal supplementation (25%) increased final body weight (1,635.4 g) and body weight gain (1,393.5 g), reduced feed intake (1,886.5 g) and FCR (1.36). Gunnal *et al*. (2006) reported that live weight gain, feed intake, feed conversion ratio and mortality were not affected by basal diet throughout supplemented probiotic (0.1% protexin) and antibiotic growth promoter (0.1% flavomycin). In contrast, Murwani *et al*. (2011) stated that body weight of broiler fed corn-mungbean basal diet supplemented yeast and noni leaf extract was lower than control.

The improvement in the body weight, daily weight gain, and feed conversion in this study may be also due to the presence of *P. acidilactici* which increased

Table 2. Growth performance of broiler offered diets with different administration of additive at 35 d of age

Observed variable	Treatments				
	A	B	C	D	E
Initial BW (g)	42.43± 0.80	42.71± 0.76	42.21± 1.05	43.46± 1.96	42.68± 0.87
Final BW (g)	1,230.28±125.44[a]	1,659.52±127.75[b]	1,121.29± 133.79[a]	1,280.56±117.27[a]	1,158.74± 63.26[a]
BWG (g)	1,187.85±125.31[a]	1,616.81±127.52[b]	1,079.08± 132.86[a]	1,237.10±117.70[a]	1,116.06± 63.13[a]
Feed intake (g)	2,782.86±263.53	3,119.24±543.02	3,125.87±1,085.95	2,662.61±250.98	2,864.70±666.27
FCR	2.27± 0.27	1.87± 0.21	2.85± 1.20	2.09± 0.20	2.49± 0.67
Mortality (%)	4.46± 3.42	3.57± 2.92	8.04± 4.49	3.57± 2.92	5.36± 4.61

Note: Means in the same row with different superscript differ significantly ($P<0.05$). A= *E. coli* infection; B= *E. coli* infection + bio additive; C= *E. coli* infection + probiotic; D= *E. coli* infection + antibiotic; E= No *E. coli* infection.

efficiency of digestion and nutrient absorption processes. Edens (2003) reported that the inclusion of desirable microorganisms (probiotics) in the diet allows the rapid development of beneficial bacteria in the digestive tract of the host, improving its performance. As a consequence, there is an improvement in the intestinal environment, increasing the efficiency of digestion and nutrient absorption processes. The beneficial effects of probiotic might also be related to general properties of probiotic such as lactic acid and enzyme production, competitive exclusion against pathogens and increase of villus height of intestine. Beside that, the improvement of performance may also due to the efficiency of undigested feed in the earthworm administration so that energy intake fully utilized by the body for life and growth as well as survival of the animals (McDonald, 2002). Earthworm meal *L. rubellus* contained 63.08% protein and 18.51% fat (of dry mass) (Damayanti *et al*., 2008) that meet the nutrition requirement of poultry. Therefore, chickens consumed smaller quantity of feed and had great growth.

Feed intake of broiler administered bio additive was not significantly different from that of broilers fed basal diets. Bio additive did not reduce the high variations in feed intake and FCR values, which might be due to the mash form of the experimental diet. Jahan *et al*. (2006) reported that broilers fed mash had lower performance than broilers fed pellet form and crumble. Salari *et al*. (2006) confirmed that chickens fed pelleted diets, consumed more feeds and showed better weight gain and FCR.

The mortality percentages of broilers with *E. coli* infection within bio additive treated group (B) and antibiotic (D) were lower (3.57%) than positive control (4.46%), negative control (5.36%) and probiotic (7.14%). Bio additive indicated an ability to reduce the negative effect of *E. coli*. Huff *et al*. (2012) reported that oral administration of probiotic reduced the mortality and increased weight gain of broiler challenged by APEC infection. Leaf extract (Deshmukh *et al*., 2011) and earthworm extracts (Cho *et al*., 1998; Tasiemski, 2008; Murwani *et al*., 2011; Damayanti *et al*., 2008; Istiqomah *et al*., 2011) exhibited antimicrobial activity against *E. coli*. Meanwhile the highest mortality rate was found in C treatment (7.14%). The mortality could be as result of disease related to *E. coli* infection and avian blood parasite (*Leucocytozoon*) decreasing the immunity of broiler due to inflammation.

Meat Quality

Fat content of broiler in bio additive, probiotic and antibiotic treatments did not significantly different from positive and negative controls (Table 3). The supplementation of bio additive, probiotic and antibiotic did not significantly affect cooking loss and water-holding capacity of meat. Ton *et al*. (2009) reported that there was no significant difference in fat content and meat quality (pH, color, cooking loss, and water-holding capacity) due to dietary supplementation of red worms. In the contrary, Kalavathy *et al*. (2006) reported that administration of *Lactobacillus* cultures (probiotic) reduced the fat carcass of broiler. Sarker *et al*. (2010) also stated that addition of *Salicornia herbacea* (Hamcho) probiotic (SHP) in broiler diet significantly lower the crude fat content in meat than control.

The value of meat pH of broiler offered probiotic and antibiotic treatments was lower (P<0.05) than positive and negative control and also bio additive treatment (Table 3). Lower pH value is often associated with an increase in meat tenderness. Non-stressed birds had meat pH in the range of 5.96 and 6.07 (Van Laack *et al*., 2000), while the meat pH in this study ranged from 6.09 to 6.24. The high value of pH caused by *E. coli* infection was likely associated with low muscle glycogen levels resulting in high meat pH (Maltin *et al*., 2003). Water-holding capacity is an important attribution of meat quality and can be measured by drip or cooking loss. Table 3 showed that the water-holding capacity and cooking loss were no significantly different among treatments. Cooking loss varied between 17.72% to 19.83%. High quality meat has low cooking loss due to less nutrients loss (Soeparno, 2005). There was no significant difference in drip loss and cooking loss due to supplemented levels of worms (Ton *et al*., 2009). The meat tenderness of broiler

Table 3. Meat quality of broiler offered diets with different administration of additive at 35 d of age

Observed variable	Treatments				
	A	B	C	D	E
Moisture (%)	71.23±0.28^{a}	71.58±0.24^{b}	70.07±0.04^{c}	70.13±0.04^{c}	72.69± 0.12^{d}
Ash (%)	3.94±0.16^{a}	3.55±0.21^{b}	3.63±0.10^{b}	3.83±0.04^{a}	4.19± 0.01^{c}
Crude protein (%)	69.13±1.45^{a}	57.85±0.36^{b}	60.41±0.47^{c}	64.70±1.97^{d}	70.14± 1.61^{a}
Crude fiber (%)	2.28±0.18^{a}	1.68±0.03^{b}	1.03±0.18^{c}	2.92±0.14^{d}	0.63± 0.02^{e}
Fat (%)	7.57±1.76	8.74±1.50	8.89±1.00	8.09±2.51	7.27± 1.26
Value of pH	6.24±0.07^{a}	6.23±0.03^{a}	6.16±0.02^{b}	6.09±0.01^{c}	6.10± 0.02^{c}
Cooking loss (%)	17.72±1.03	18.41±1.92	19.83±1.68	18.48±1.66	19.04± 0.90
Water-holding capacity (%)	32.98±5.42	34.42±3.14	31.61±4.36	30.11±6.63	36.24±11.90
Tenderness (kg.cm^{2})	5.79±0.07^{a}	5.77±0.03^{a}	5.71±0.02^{b}	5.64±0.01^{c}	5.65± 0.02^{c}

Note: Means in the same row with different superscript differ significantly (P<0.05). A= *E. coli* infection; B= *E. coli* infection + bio additive; C= *E. coli* infection + probiotic; D= *E. coli* infection + antibiotic; E= No *E. coli* infection.

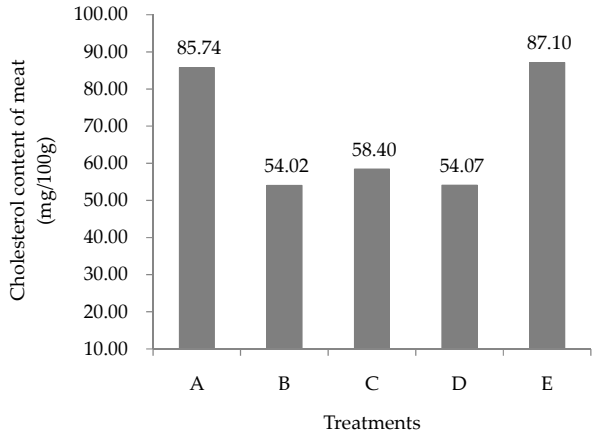

Figure 1. Cholesterol contents of broiler meat as responses to diets administered with bio additive, probiotic, and antibiotic into drinking water. A= *E. coli* infection; B= *E. coli* infection + bio additive; C= *E. coli* infection + probiotic; D= *E. coli* infection + antibiotic; E= No *E. coli* infection.

administered probiotic and antibiotic was lower (P<0.05) than positive control and bio additive. Lyon *et al*. (2004) reported that tenderness of broiler chickens ranged between 1.82 to 2.19 kg/cm^2, while broiler meat tenderness in the present experiment ranged from 5.64 to 5.79 kg/cm^2. The level of meat tenderness due to the administration of bio additive was associated with the value of pH. Meat with high value of pH was more juice and more tender (Soeparno, 2005). Zhu *et al.* (2010) reported that broilers muscle tenderness and cooking loss were not affected by earthworm administration. Fanatico *et al*. (2007) stated that drip loss and cooking loss were high in the slow-growing birds but low in the fast-growing and medium-growing birds.

Effect of bio additive, probiotic, and antibiotic administration into drinking water on cholesterol content is presented in Figure 1. Broilers given bio additive, probiotic, and antibiotic treatments produced meat with lower cholesterol content (P<0.05) than positive and negative control. The lowest meat cholesterol was obtained with administration of bio additive (54.02 mg/100 g). The mechanism responsible for the cholesterol-lowering effect of probiotics remains unclear, but it has been suggested that the effect could be obtained through retarded cholesterol synthesis in the gastro-intestinal tract by probiotic supplementation and increased degradation of cholesterol. It was speculated that *Lactobacillus acidophilus* reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis (Abdulrahim *et al*., 1996). *Lactobacillus* has found to have a high bile salt hydrolytic activity, which is responsible for deconjugation of bile salts (Surono, 2003). Administration of *Lactobacillus* cultures (LC) on broiler reduced the cholesterol content of carcass by 13%, since *Lactobacillus* cultures produced bile salt hydrolase and exhibit de-conjugating activity of bile salts, which contributed to an increased in excretion of cholesterol and reduced cholesterol in the meat of chickens (Kalavathy *et al*., 2006). Alkhalf *et al*. (2010) reported that chicken fed a diet containing probiotic had low meat cholesterol and serum (Taheri *et al*., 2010). Administration of fermented noni leaf reduced the cholesterol content of broiler carcass which was due to the increased in β-carotene intake (Syahruddin *et al*., 2011). The more β-carotene in consumption, the lower the cholesterol content of the carcass due to β-carotene inhibited Hydroksimetyl glutaryl-CoA reductase, reduced the formation of mevalonic and therefore reduced cholesterol synthesis. Murwani *et al*. (2011) also reported that serum cholesterol of broiler fed diet supplemented by combined baker yeast and noni leaf extract was low.

CONCLUSION

Administration of bio additive or mixed earthworms extract, noni leaves extract and lactic acid bacteria increased the growth performance, feed efficiency, and reduced meat cholesterol content of broiler infected with *E. coli*.

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